IMPEDANCE MEASUREMENT SYSTEM FOR EMBRYONIC STEM CELL AND EMBRYOID BODY CULTURES

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IMPEDANCE MEASUREMENT SYSTEM FOR EMBRYONIC STEM CELL AND EMBRYOID BODY CULTURES

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SUMMARY

The objective of the proposed research is to design an experimental setup to assess the ability of impedance measurements to characterize mouse embryonic stem cell (ESC) and embryoid body (EB) growth and differentiation. Existing quality assurance measurements used to stage the growth and differentiation of embryoid bodies are labor intensive and most often destructive to the cells, thus present methods are typically valid for a single time point. Bioimpedance measurements are non-invasive and non-destructive, presenting an alternative approach to this challenge. These measurements can be done continuously for real-time measurements on the changes in embryoid body growth and differentiation.

A system capable of making bioimpedance measurements of ESC and EB suspensions was designed along with a biocompatible test device to hold the cells and Ag-AgCl electrodes. The system uses a lock-in amplifier to record the magnitude and phase changes of the ESC and EB suspensions when a 1 Vpp signal sweeping frequencies from 100 Hz to 100 kHz is applied. The system performance was validated with a test case of 1 mL of 0.1 M KCl. Then experiments with cell culture media, ESCs, and EBs were performed, with varying concentrations of cells and EBs.

Experimental results for single ESC suspensions showed promise in detecting a difference in cell concentration between 2 million and 4 million cells in 0.5 mL of media. Results for four day old EBs were ambiguous, and we conclude that a different experimental set up is required due to EB settling during experimentation.

CHAPTER 1

INTRODUCTION

Recently there has been a large investment in research regarding embryonic stem cells (ESCs). Embryonic stem cells are appealing for cell-based therapies, such as tissue regeneration, because of their ability to self-renew, and they can be differentiated into different types of tissues. ESCs can be grown in culture and differentiated as embryoid bodies (EBs), which are 3D aggregates of embryonic stem cells. One research challenge regarding experimental culturing of ESCs and EBs is control of their growth and differentiation. Current methods for determining cell size, number and state of differentiation include polymerase chain reaction (PCR), histology and flow cytometry. A better direct method for characterization of growth and differentiation is needed because both PCR and histology are destructive methods, requiring simultaneous batches of cells to be grown in order to test their progress. Flow cytometry is not destructive to the cells, but it requires dissociating EBs. Another disadvantage is that none of these methods are a continuous or real-time measurement. An ideal measurement system would measure the point in time when the ESCs start to form EBs, or when EBs start to differentiate into tissue, and ideally could distinguish what type of tissue the EBs are forming. Bioimpedance measurement (BIM) is a non-invasive, non-destructive method to characterize tissues, and can also be done in real-time as a continuous measurement. Current research has shown that BIM can accurately measure cell shape and growth [1-3], cell motion [5-9], and heights of 3D cell cultures [10].

Bioimpedance is the measurement of opposition to alternating current flow in tissue and is comprised of a constant and time-varying component, resulting in a complex impedance

characteristic, $Z = \frac{V}{I}$ [11,12]. Over a range of frequencies the magnitude and phase response can be examined and has been found to be unique to different types of biological tissues. Typically, the unique impedance response can be modeled by an equivalent circuit, and the most basic equivalent circuit is a resistor and capacitor in series. However, to be more accurate, the resulting impedance characteristic can not be described by simple RC circuits and a combination of them must be used. A major challenge of this project is determining the extent to which BIM models, which are usually applied to bulk tissue, can be applied to individual cell suspensions and cell aggregates in a fluid media, and which equivalent model is appropriate.

In the following chapters the system design, experimental set up, and protocol will be presented to acquire BIM of ESCs and EBs suspended in culture media. Next, a test case that validates the system functionality and the experimental results of the undifferentiated media, single cell suspensions of ESCs and Day 4 EBs will be presented and evaluated. Finally, the work will be summarized and future work will be proposed for the continuation of the project.

CHAPTER 2

SYSTEM DESIGN

The primary goal of this project was to design a system to determine if impedance measurements can characterize ESC growth and EB growth and differentiation. First, a feasible and reliable experimental setup including data acquisition and data analysis scheme must be designed. This setup must be biocompatible to hold the cells, with electrodes that are robust and do not introduce error to the system, such as noise. Current and voltage electrode placement should be fixed to ensure reproducibility of measurements. The acquisition limitations must be known to determine the maximum allowable frequency to test the cell cultures. Analysis calculations must be dependable and correct when looking at the magnitude and phase change over the frequency sweep. And finally, the results can be interpreted to see whether impedance measurements can characterize ESCs and EB growth and differentiation.

2.1 Test Device Design

A device was fabricated to hold the cell suspensions for experimental testing. This device had to be biocompatible, easy to use, and have fixed positions for electrode placement to ensure reproducible experimental measurements. Figure 1 shows a top and side view, as well as a view of the channel of the device fabricated. It was machined out of polycarbonate using a computer numerical control (CNC) mill. The device is made up of two pieces, a bottom piece with a channel to hold the cell culture and a top piece that is a lid over the channel with designated slots to hold the Ag-AgCl electrodes. Dimensions of the channel are 9.7425 cm x 0.3175 cm x 0.508 cm, holding a maximum 1.5 mL of solution. The electrode slots are spaced

5.08 mm apart at the closest points, and 12.7 mm apart between electrode points 2, 3, 5, 7, 9, 11, 13, and 15.

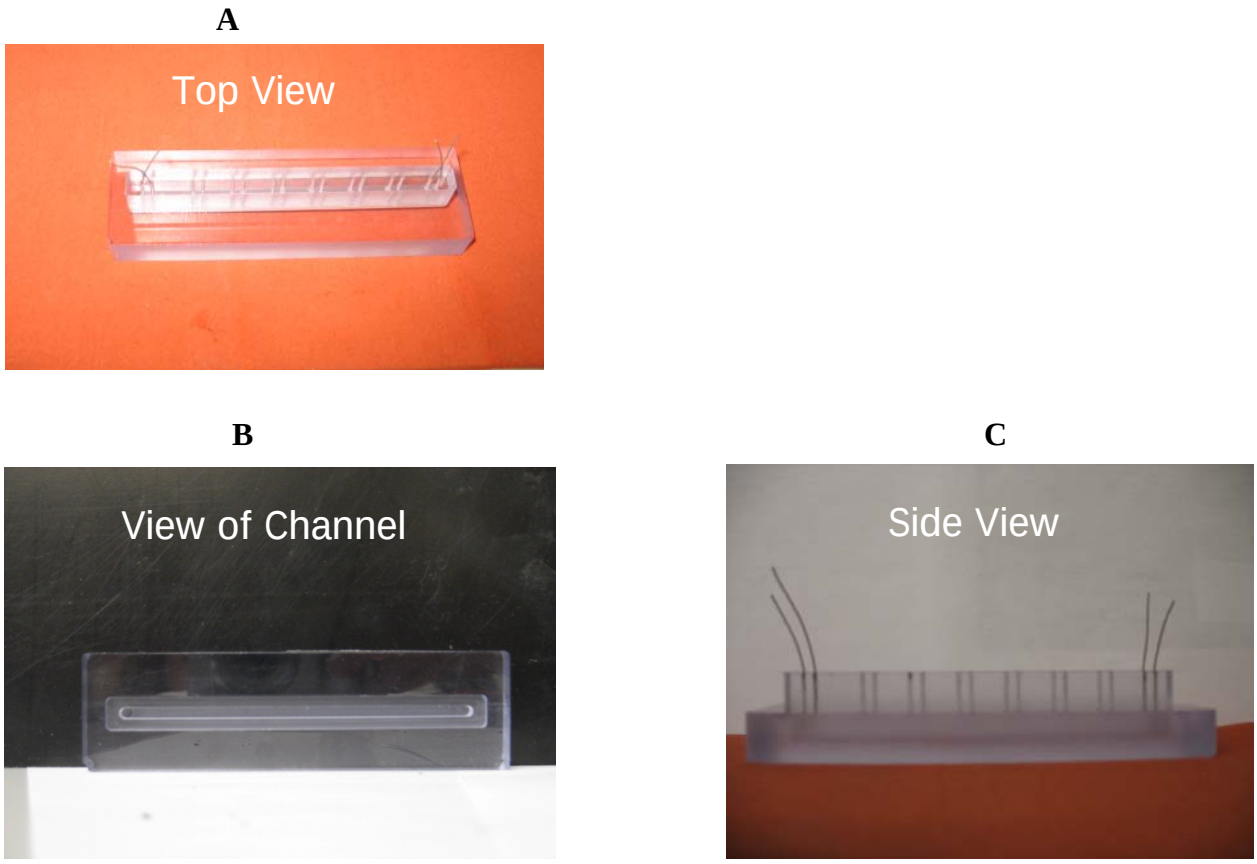

Figure 1 Views of the testing device A) top view, B) view of channel, C) side view.

2.2 Experimental Set Up

Bioimpedance is the measurement of the opposition of current flow in tissue and can be calculated as $Z = \frac{V}{I}$. BIM can be made one of two ways, a current can be injected in the tissue and a voltage drop is then measured, or a voltage is set across the tissue and the resulting current is measured [11,12]. Multiple electrode configurations to make BIM can be used. Two possible arrangements are bipolar or tetrapolar. A bipolar configuration uses only two electrodes and

takes both the current and voltage measurements from them, while a tetrapolar configuration utilizes four electrodes, two of which are current injecting electrodes and the other two are voltage sensing electrodes. The electrode configuration must be determined and accounted for when processing the BIM results.

2.2.1 Initial Experimental Set Up

The initial test set up used a tetrapolar arrangement, with two current injecting electrodes and two voltage sensing electrodes, placed inside the current sensing electrodes as seen in Figure 2. A Howland current source was used as the constant current source injecting the current into the cell culture. A LabView program was designed to control the function generator and acquire the data with the NI-DAQ SC-2040 and PCI-6429 board. The LabView program performed a frequency sweep from 100 Hz – 100 KHz spaced logarithmically, injecting a current of 15 μA with a sampling frequency of 500 kHz. The injected current level of 15 μA was chosen because when ~300 mV is applied to a cell membrane, it starts to perforate. Once the data was acquired, a Matlab program used a non-linear least squares curve-fit to optimize the magnitude and phase information between the stimulus and response for each known frequency. From there, the response was displayed for interpretation in both a polar plot and a magnitude-phase plot.

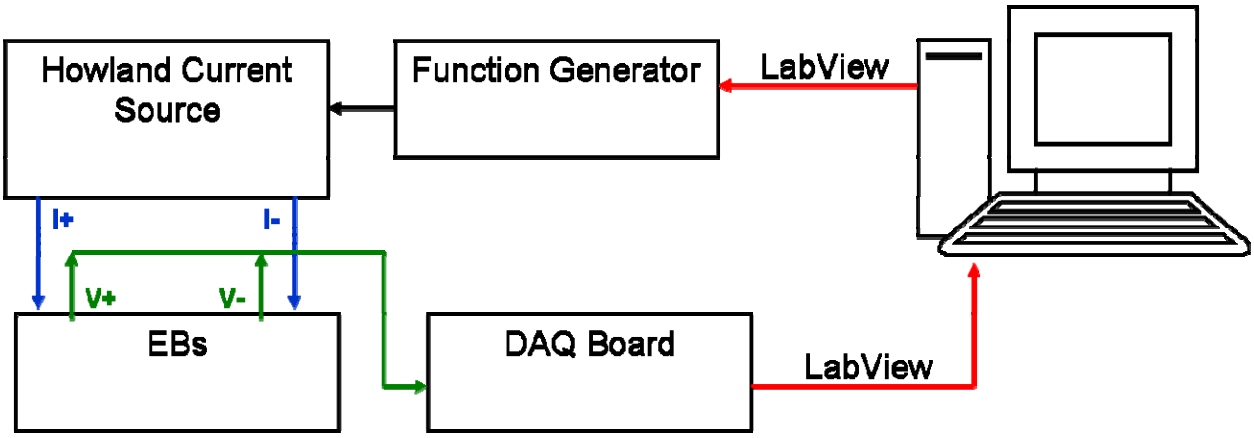

Figure 2 Initial System Block Diagram.

The non-linear least squares curve-fit relied on the assumption that the measured signal at a given frequency was only shifted in time and changed in magnitude. The effects of measurement noise along with limitations in the sampling frequency of the NI-DAQ SC-2040 led to errors in the post processing. The frequency limits of the current source may have also hampered measurements. As a result, a new testing system was used.

2.2.2 Present Experimental Set Up

In order to accurately measure the magnitude and phase change of the ESC and EB impedance response, the new experimental set up utilizes a lock-in amplifier. A lock-in amplifier outputs a signal at a given frequency and then can "lock-in" on that frequency and detect the magnitude and phase change of the transformed signal from the cell culture. The lock-in bypasses the post-processing and sampling problems faced in the initial set up. This new experimental set up, shown in Figure 3, employs a bipolar electrode arrangement, inputs voltage, and then measures the current flowing through the cell culture with a voltage-to-current (VI) converter. A 1 Vpp signal is input to the cells with a frequency sweep of 50 evenly spaced

frequencies between 100 Hz and 100 kHz. The lock-in amplifier records the magnitude and phase shift of the ESC and EB suspensions. This system is run by a program written in Matlab.

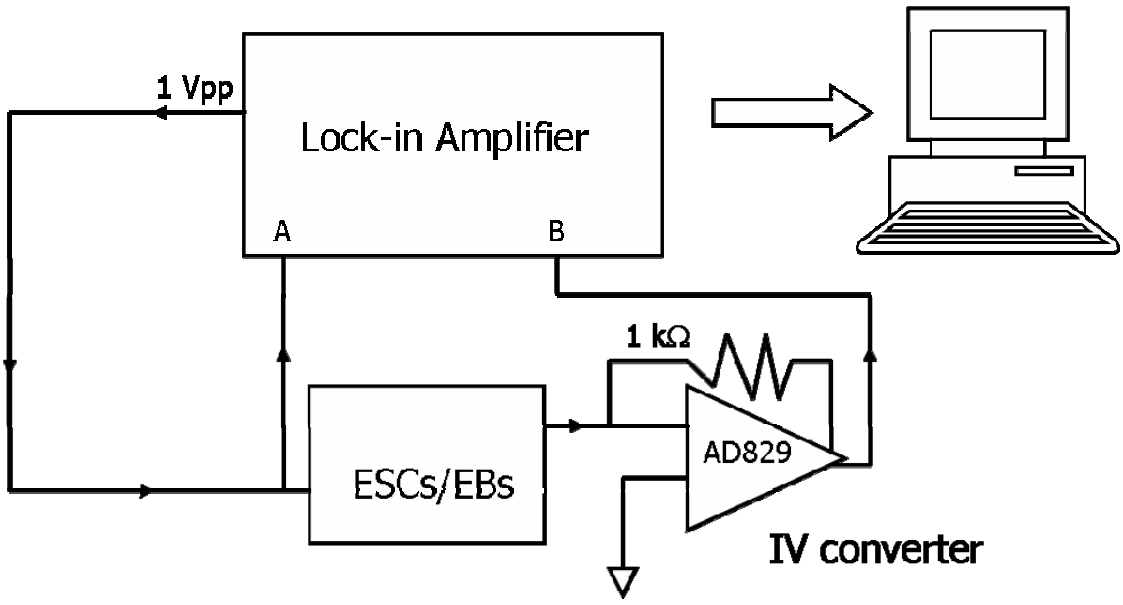

Figure 3 Present Experimental Set Up.

2.2.3 Test Case – 0.1 M KCl

In order to prove reliability and functionality of the experimental setup, a known quantity must be measured. Experiments were conducted with 1 mL of 0.1 M KCl. From the known conductivity of 0.1 M KCl of 1.282 S/m [13], and knowing the dimensions of the test device, the expected resistance could be calculated, as shown below, resulting in an expected resistance, R_{KCl}, of 6824.4 Ω.

$$R = \frac{\rho L}{A} = \frac{L}{\sigma A}$$

$$\sigma = 1.28217\ S/m \qquad L_{channel} = 9.8425\ cm \qquad L_{measurement} = 8.89\ cm$$

$$A_{solution} = \frac{Volume\ Solution}{Length\ Channel} = \frac{1\ mL}{9.8425\ cm} = 0.1016\ cm^2$$

$$R_{KCl} = \frac{L_{measurement}}{\sigma A_{solution}} = \frac{8.89\ cm}{1.28217\ S/m \quad * \quad 10^{-2}\ cm^{-1} \quad * \quad 0.1016\ cm^2} = 6824.4\ \Omega$$

Figure 4 shows the magnitude and phase plot of four trials of 1 mL 0.1 M KCl and Figure 5 shows the polar plot of the frequency sweep. These figures show the measured impedances, $Z_{EXP\ KCl}$, made up of the impedance of the electrodes, $Z_{ELEC,}$ and KCl solution, Z_{KCl}. As mentioned earlier, this is due to the bipolar electrode arrangement used in this system design. Figure 7 shows Z_{ELEC}, measured when the electrodes were placed 5.08 mm apart. Assuming the impedance of KCl and electrodes can be modeled as two impedances in series, Z_{KCl} can be calculated as $Z_{KCl} = Z_{EXP\ KCl} - Z_{ELEC}$. Z_{ELEC} was only recorded in Trial 4 and 5, so for the other trials the average of this measurement was used to calculate Z_{KCl}.

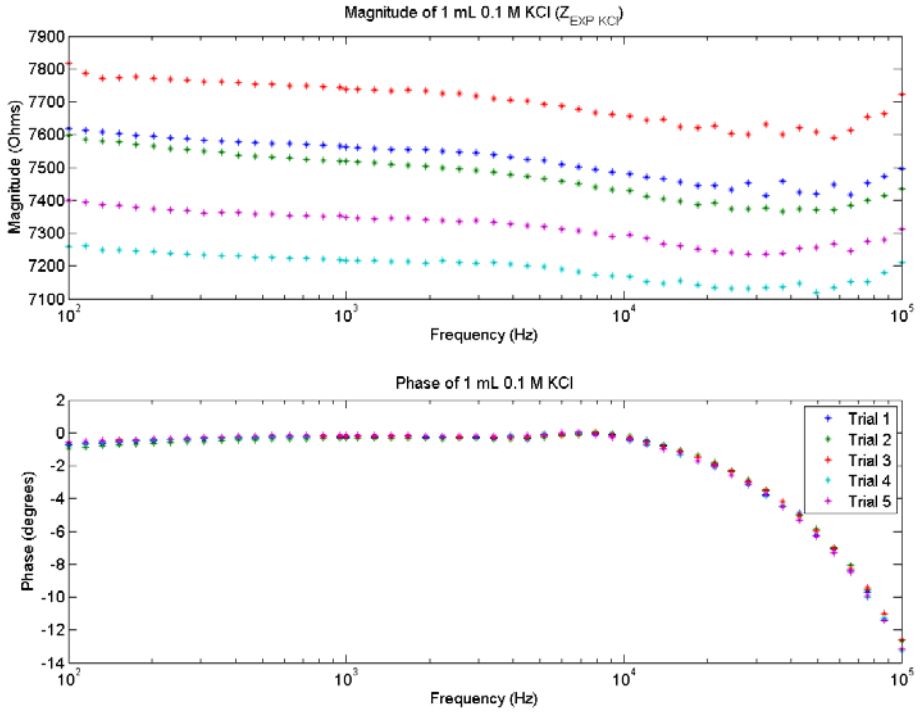

Figure 4 Experimentally Measured Magnitude and Phase Impedance Response of 1 mL 0.1 M KCl ($Z_{EXP\ KCl}$).

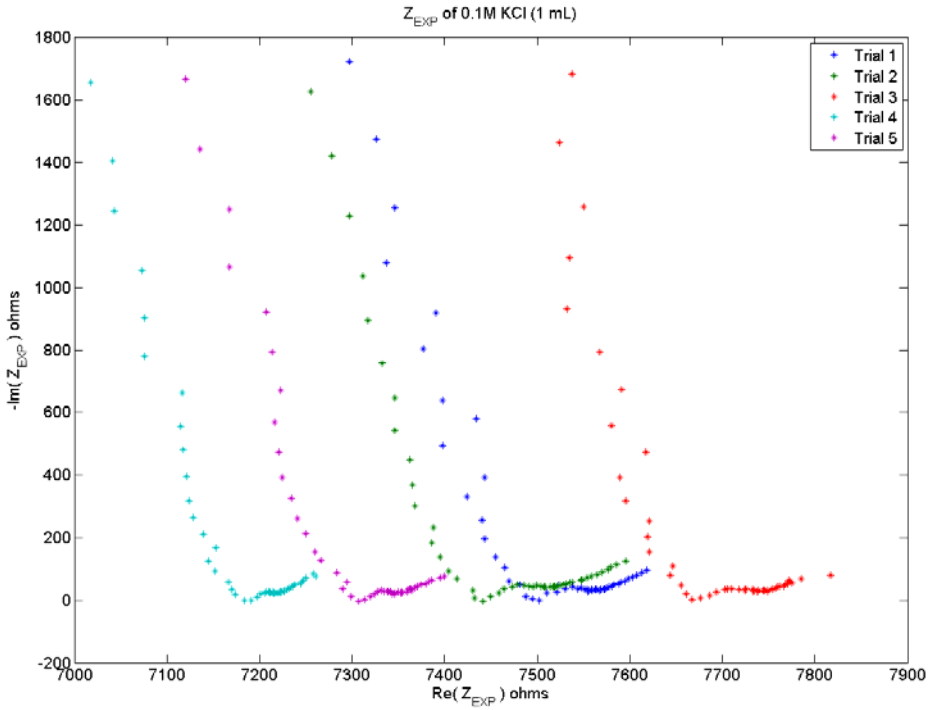

Figure 5 Experimentally Measured Complex Impedance Response of 1 mL 0.1 M KCl ($Z_{EXP\ KCl}$).

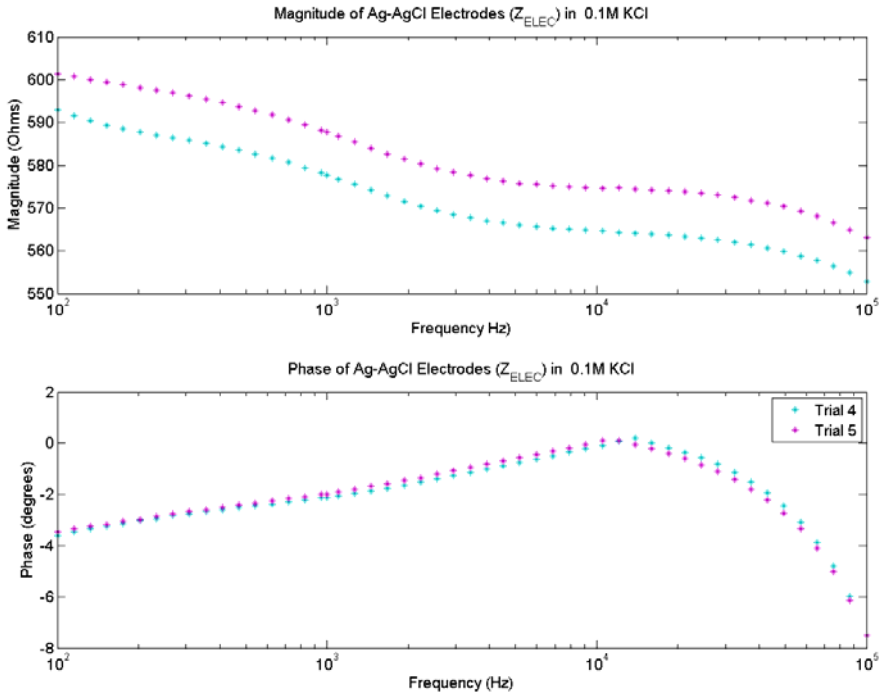

Figure 6 Measured Magnitude and Phase Impedance Response of Ag-AgCl Electrodes in 1 mL KCl.

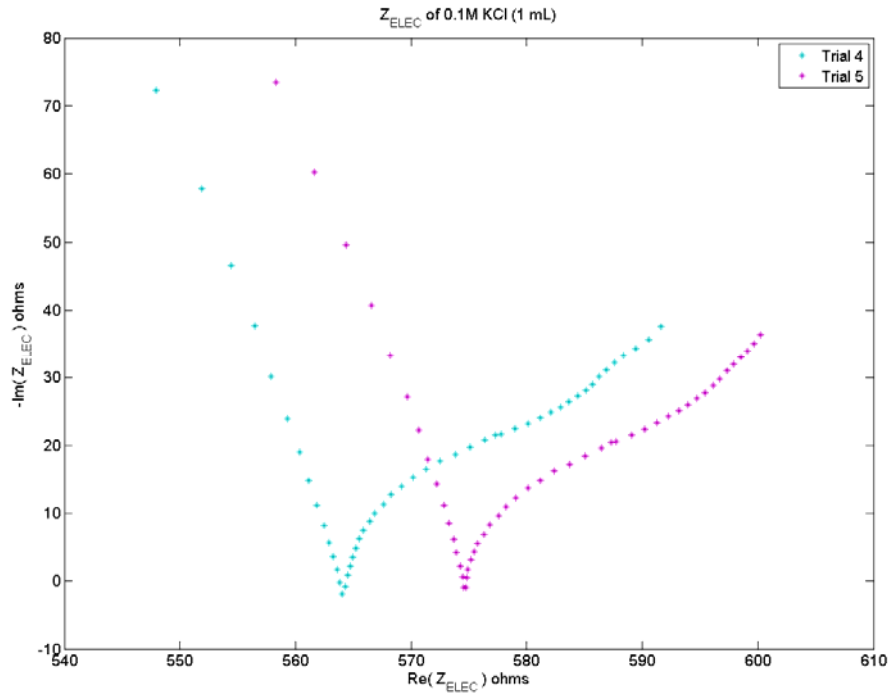

Figure 7 Complex Impedance Response of Electrodes (Z_{ELEC}).

Figure 8 and Figure 9 show the calculated magnitude and phase response of Z_{KCl} and the calculated complex impedance response respectively. The theoretical R_{KCl} of 6824.4 Ω is also shown in Figure 8 and Figure 9 for reference with the yellow trace. Figure 8 shows that the magnitude of Z_{KCl} ranges from 6600 Ω to 7300 Ω, encompassing the expected impedance of 6824.4 Ω. The phase change dropping from -1° to -13° after 10 kHz, seen in Figure 4 and Figure 8, is an artifact of the lock-in amplifier and VI converter. It is not rectified for because the phase roll-off of the experimental measurements and the electrode measurements have different phase responses.

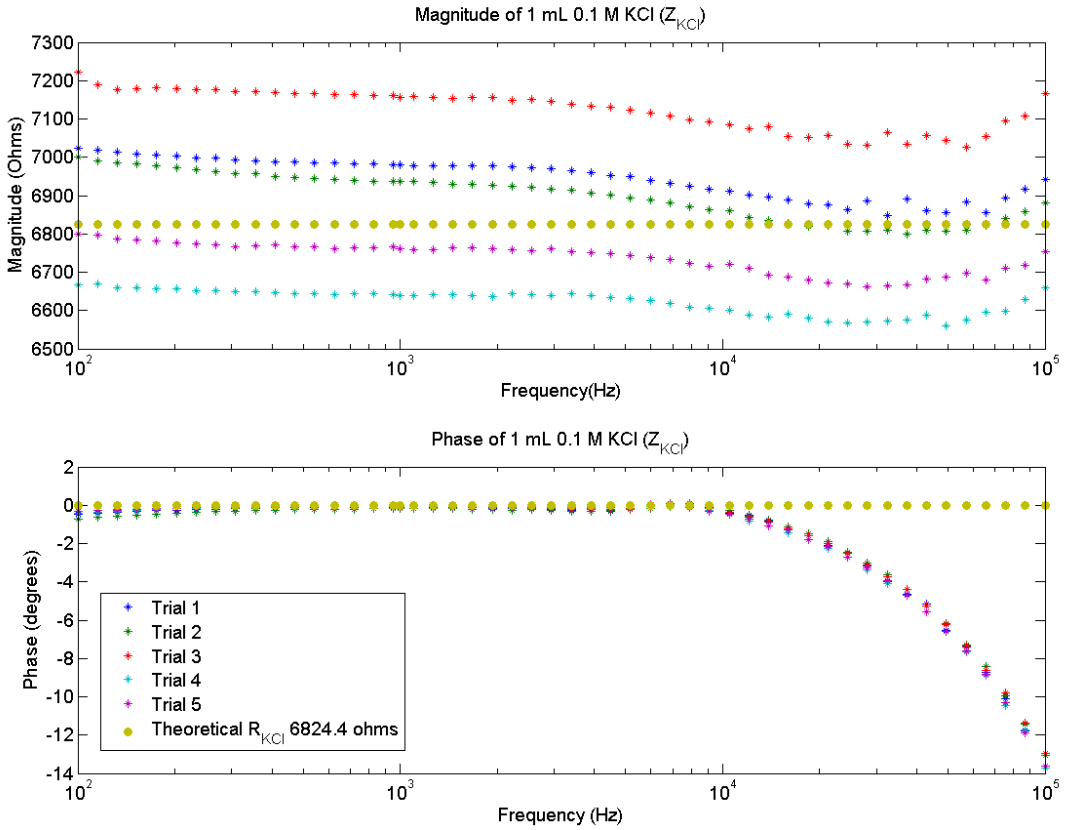

Figure 8 Calculated Magnitude and Phase Impedance Response of 1 mL 0.1 M KCl (ZKCl).

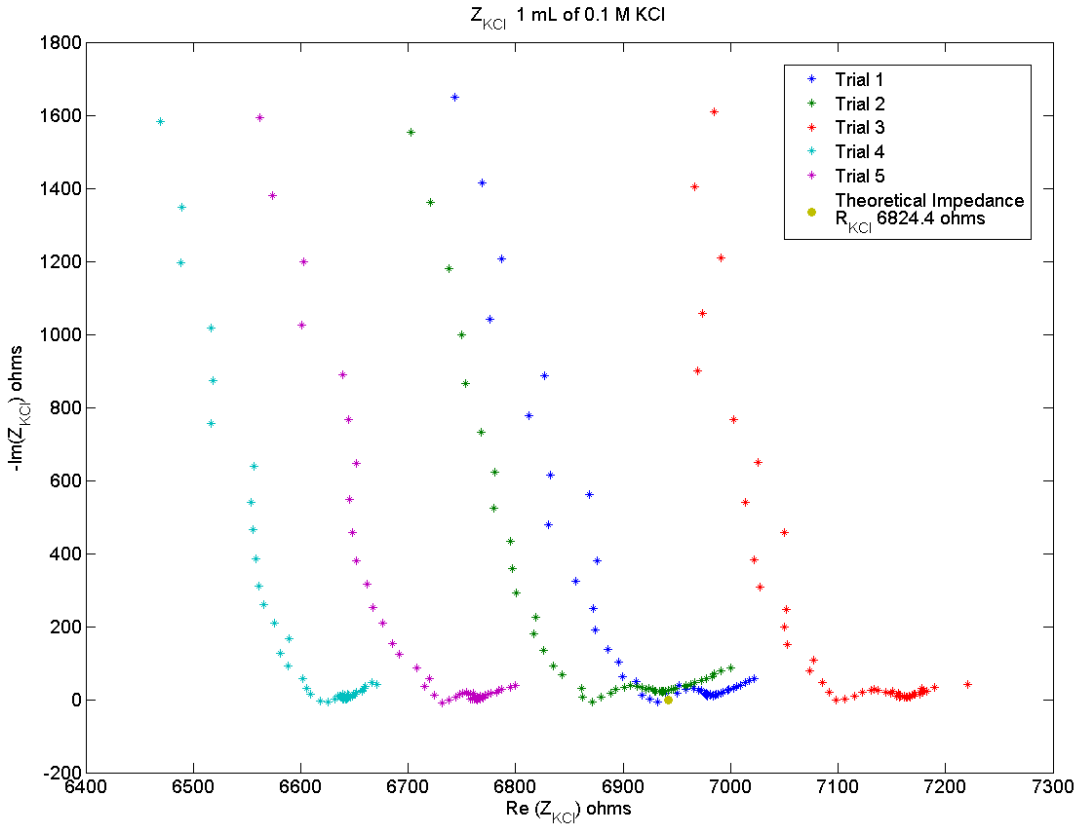

Figure 9 Calculated Complex Impedance Response of 1 mL 0.1 M KCl (Z_{KCl}).

Table 1 shows the magnitude of Z_{KCl} at 100 Hz, taken from Figure 8, and their corresponding conductivities, $\sigma_{Measured\ KCl}$, and compares them to the known conductivity of 1.28217 S/m [13]. The mean measured conductivity, $\sigma_{Mean\ KCl}$, is 1.2613 S/m, with a standard deviation of 0.039 and a standard error of 0.0174. Standard error was calculated as $SE = \frac{std}{\sqrt{N}}$, where std is the standard deviation and N is the sample size. With such a small standard error, the results confirm that the present experimental system can accurately measure 1 mL of 0.1 M KCl solution, thus further measurements can be accepted as valid.

Table 1 Theoretical Conductivity of 0.1M KCl Compared to Measured Conductivity at 100 Hz.

	$\lvert Z_{KCl} \rvert$ Ω	$\sigma_{Measured\ KCl}$
Trial 1	7022.5	1.2460
Trial 2	7000.6	1.2499
Trial 3	7221.1	1.2117
Trial 4	6666.4	1.3126
Trial 5	6799.4	1.2869

Theoretical σ (S/m)	Mean $\sigma_{Measured\ KCl}$ (S/m)
1.28217	1.2614
Standard Deviation $\sigma_{Measured\ KCl}$	Standard Error $\sigma_{Measured\ KCl}$
0.0391	0.0175

2.2.4 Experimental Protocol

This project focuses on looking at the impedance response of ESCs and EBs at different points in time and in different concentrations of cells. In order to have comparable results, a standard measurement protocol must be used. Since the cells are suspended in media, a baseline measurement of the media alone must be taken to see how its impedance response compares to the response of media with cells in it. Along with a baseline media measurement, seeing as a bipolar electrode setup is being used, the impedance of the electrodes must be accounted for. Therefore multiple impedance measurements were taken for each trial of ESCs or EBs. Figure 10 depicts the experimental measurement protocol. First a measurement, Z_{DMEM}, of 0.5 mL of Dulbecco's Modification of Eagle's Medium (DMEM) was taken, with the electrodes 8.89 cm apart. Then a measurement of 0.5 mL of DMEM with a concentration of cells suspended was taken, Z_{EXP}, also with the electrodes 8.89 cm apart. A third measurement, Z_{ELEC} is taken with the electrodes very close together, only 5.08 mm apart. With the electrodes this close together it is assumed that Z_{ELEC} is dominated by the electrode impedance and the impedance from the media

and cells is negligible. From these three measurements, the impedance response of ESCs or EBs can be investigated.

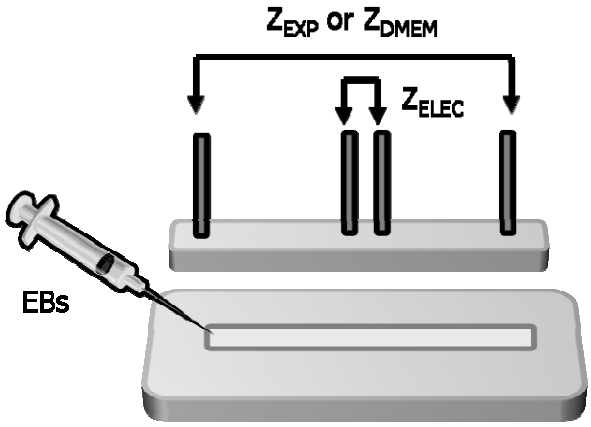

Figure 10 Experimental Measurement Protocol.

2.2.5 Culturing ESCs and EBs

Mouse (D3 line) ESCs were cultured on 0.1 % gelatin-coated tissue culture polystyrene dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal bovine serum (FBS), 2mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomyocin, 0.25 μg/mL amphotericin, 1x MEM nonessential amino acid solution, 0.1 mM 2-mercaptoethanol. 10^3 U/mL leukemia inhibitory factor (LIF) was added to the media solution upon each re-feeding maintaining the ESCs in an undifferentiated state. The cells were passaged every 2-3 days before reaching 70% confluency.

In order to differentiate the cells as EBs, the undifferentiated ESCs were dissociated from their culture with 0.05% trypsin-EDTA solution, and resuspended in media. Each Petri dish of EBs started with 2 x 10^6 undifferentiated stem cells in 10 mL of media. The EB dishes were then placed on an rotary orbital shaker, rotating at 40 rpms in the incubator. It has been shown that

rotating the EB dishes while culturing can enhance uniformity of EB yield and formation [14]. Figure 11 shows phase images of EBs at days 2 and 5 of differentiation. The scale bars in each image are 200 μm. As the EBs differentiate, they become larger in size and more opaque in appearance.

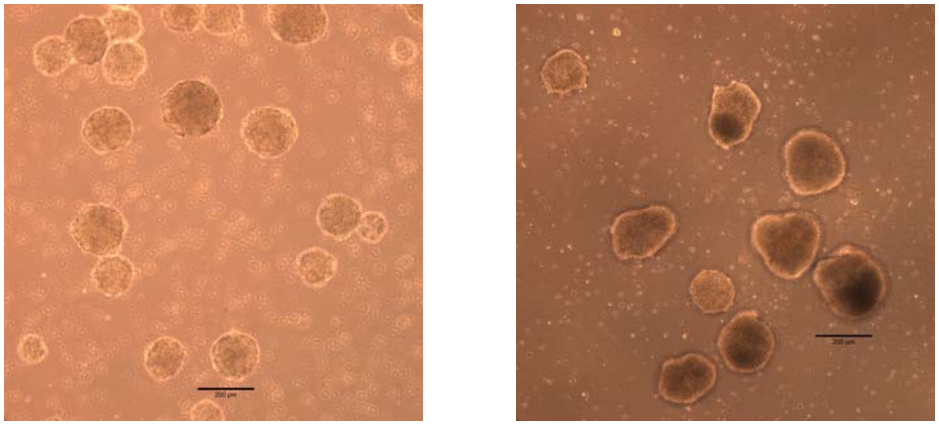

Figure 11 Phase Images of Day 2 (left) and Day 5 (right) EBs.

CHAPTER 3

EXPERIMENTS

3.1 Baseline Measurements (DMEM)

As mentioned earlier, baseline measurements of the cell media must be taken in order to detect a change after ESCs or EBs are added to it. Multiple trials of 0.5 mL of the undifferentiated media (DMEM) were conducted measuring $Z_{EXP\ DMEM}$. For each trial fresh media (0.5 mL) was measured. Based on the same assumptions used in the 0.1M KCl measurements, $Z_{MEDIA} = Z_{EXP\ DMEM} - Z_{ELEC}$, so two measurements were taken, $Z_{EXP\ DMEM}$ and Z_{ELEC}. Figure 12 and Figure 13 show the magnitude and phase response, and the complex impedance response of $Z_{EXP\ DMEM}$, respectively. Figure 14 shows the complex impedance response of Z_{ELEC}.

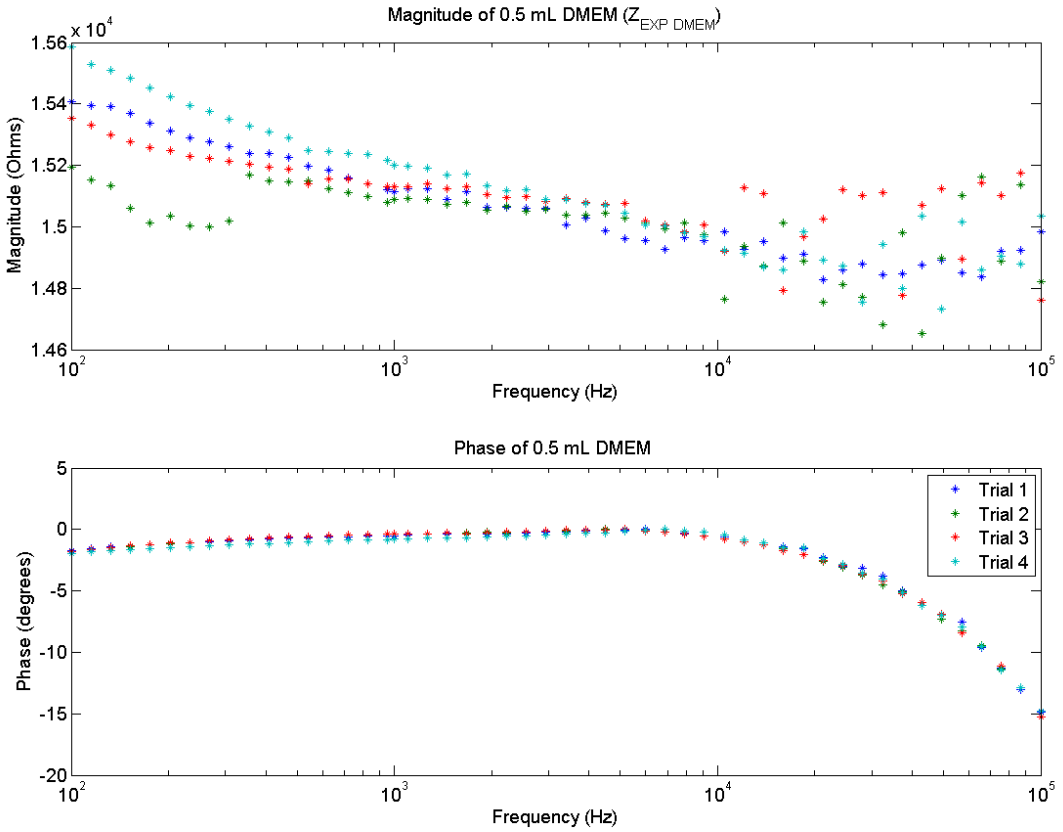

Figure 12 Measured Magnitude and Phase Impedance Response of 0.5 mL DMEM ($Z_{EXP\ DMEM}$).

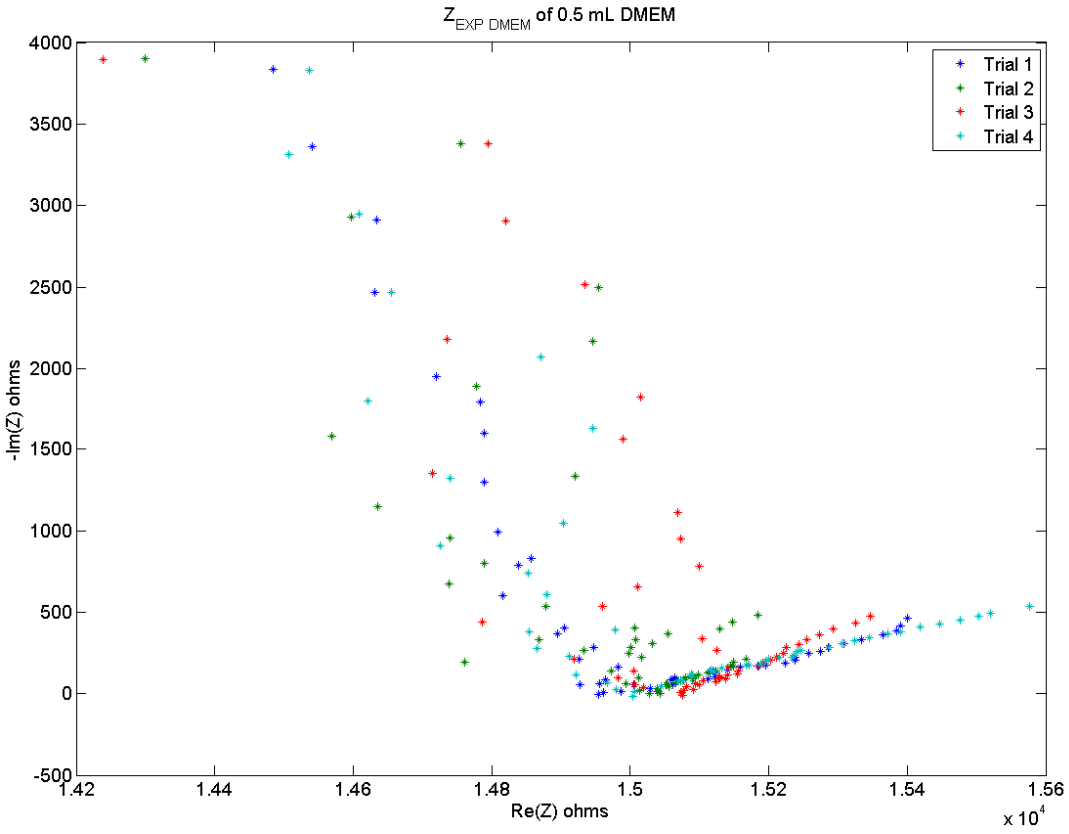

Figure 13 Measured Complex Impedance Response of 0.5 mL DMEM ($Z_{EXP\ DMEM}$).

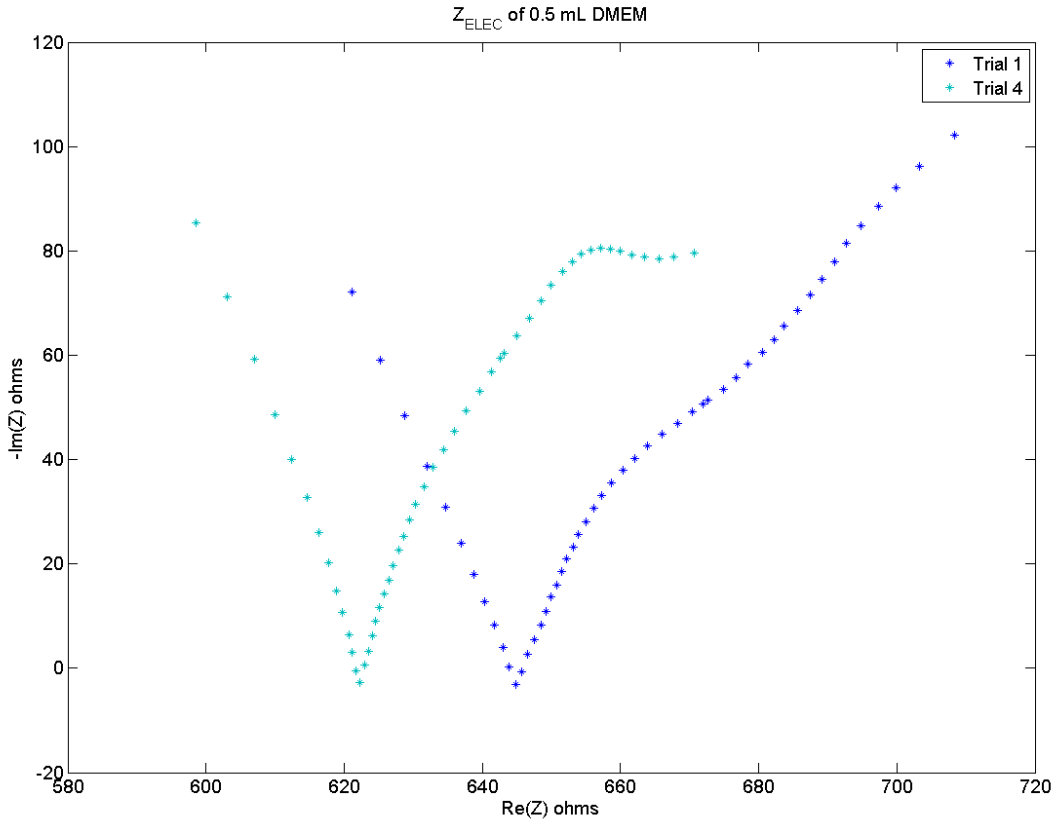

Figure 14 Complex Impedance Response of Z_{ELEC}.

Z_{MEDIA} was calculated using the equation above and since Z_{ELEC} was only measured for trial 1 and 4; the average of Z_{ELEC} was used to calculate Z_{MEDIA} for the remaining trials.
Figure 15 shows the calculated magnitude and phase response of Z_{MEDIA} and Figure 16 shows the complex impedance response of Z_{MEDIA}. In
Figure 15 the magnitude of Z_{MEDIA} ranges from approximately 14.5 kΩ to 14.9 kΩ at 100 Hz and decreases to a range of 14.2 kΩ to 14.6 kΩ at 100 kHz. The phase goes from approximately -1 degrees to -15 degrees, but this type of phase change is consistent with that seen with the KCl measurements.

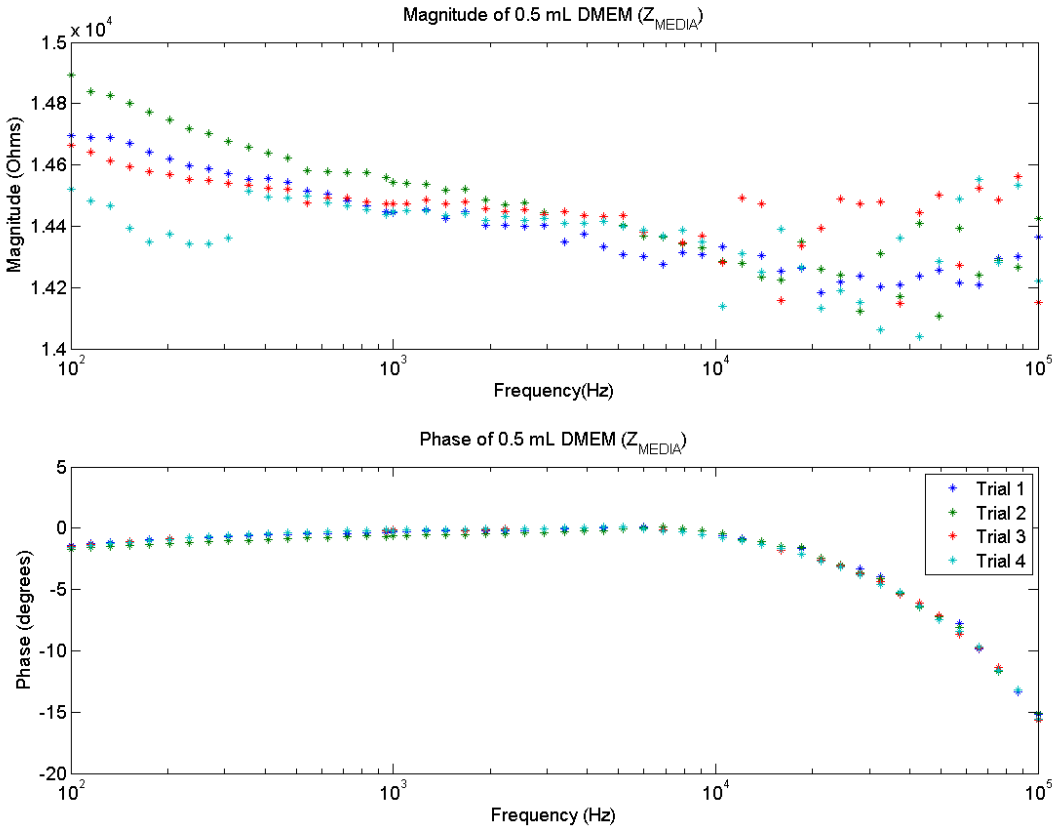

Figure 15 Calculated Magnitude and Phase Impedance Response of 0.5 mL DMEM (Z_{MEDIA}).

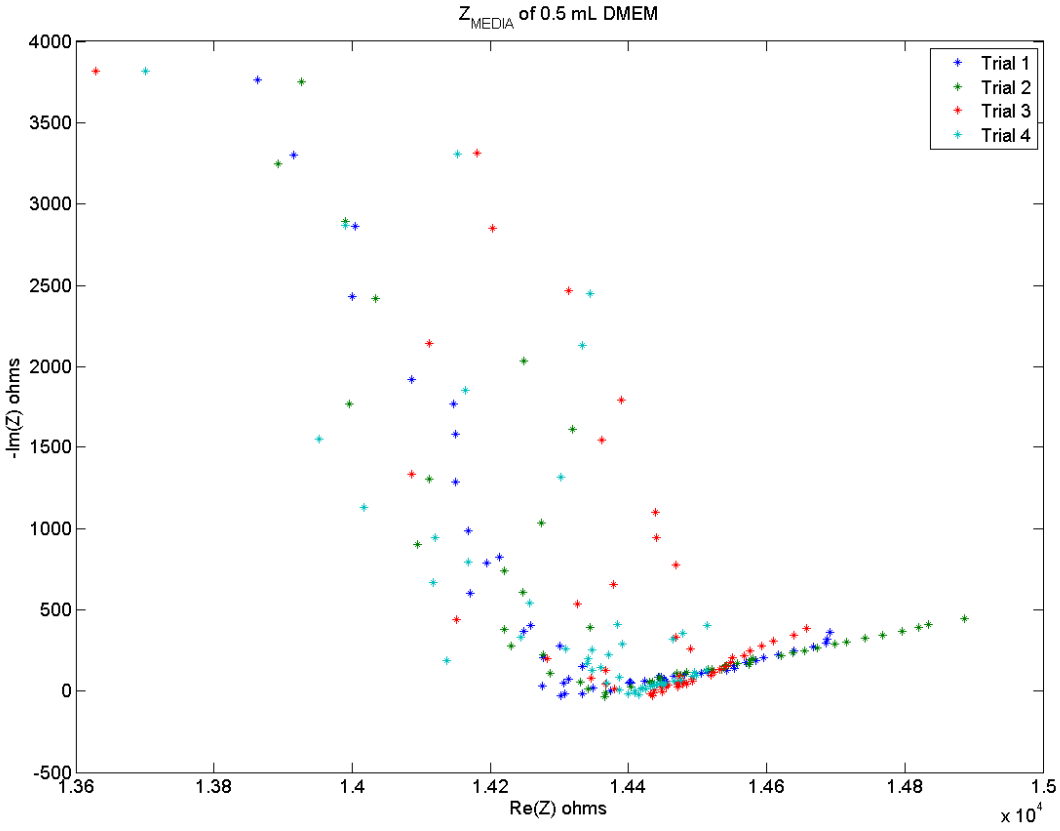

Figure 16 Calculated Complex Impedance Response of 0.5 mL DMEM (Z_{MEDIA}).

Table 2 shows the magnitude of Z_{MEDIA} at 100 Hz and their corresponding calculated conductivities. The mean conductivity of the DMEM media is 0.5959 S/m with a standard deviation of 0.00586 and a standard error of 0.00293.

Table 2 Measured Conductivity of 0.5 mL DMEM at 100 Hz.

	$\lvert Z_{MEDIA} \rvert$ kΩ	$\sigma_{Measured\ Media}$ (S/m)
Trial 1	14.697	0.5954
Trial 2	14.893	0.5875
Trial 3	14.662	0.5968
Trial 4	14.520	0.6026

Mean $\sigma_{Measured\ Media}$ (S/m)	Standard Deviation $\sigma_{Measured\ Media}$	Standard Error $\sigma_{Measured\ Media}$
0.5956	0.00622	0.00311

3.2 Single Cell Suspensions

The first set of experiments measured single cell suspensions of ESCs. Three trials were conducted. The first two trials had 2 million ESCs suspended in 0.5 mL of undifferentiated media, while the third trial had 4 million ESCs. The three measurements of Z_{DMEM}, Z_{EXP}, and Z_{ELEC} were taken. Figure 17 displays the experimentally measured complex impedance responses of each trial (Z_{EXP}) and Z_{DMEM}. Figure 18 displays the normalized real and imaginary impedance responses of each trial, Z_{EXP} / Z_{DMEM}. Normalizing the impedance responses looks at what overall effect the cells have compared to media alone. The stars represent the real normalized response and circles represent the imaginary normalized response. It can be seen that while the real responses do not change significantly between trials, i.e. change in cell concentration, the imaginary response of the 4 million single cell suspension (SS 4 million) response differs from the 2 million single cell suspension (SS 2 million) response. The magnitude of the imaginary response of 4 million cells decreases faster than the 2 million cells. At 720 Hz, the Im(SS 4 million) is 0.4703 while Im(SS 2 million Trial 1) is 0.7672 and Im(SS 2 million Trial 2) is 0.7941. The imaginary response can be attributed to the capacitive behavior of the cells, and thus the results show promise that a change in cell density can be detected with this system.

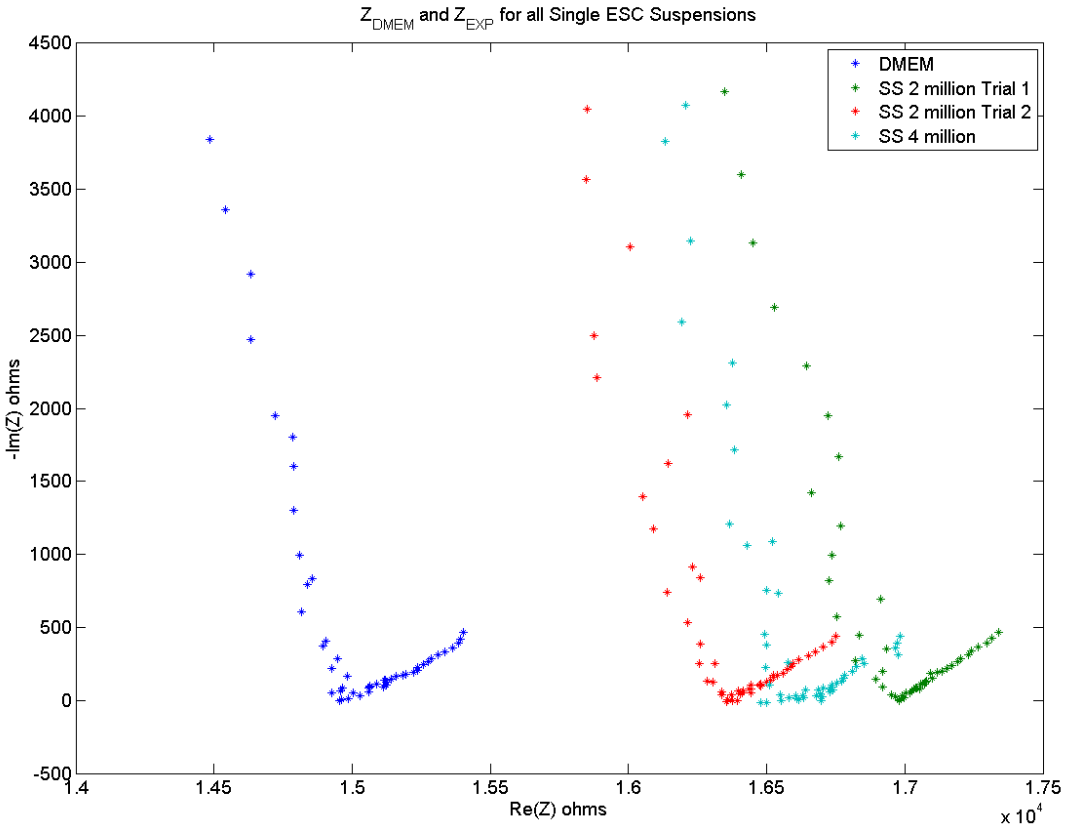

Figure 17 Complex Impedance Response of DMEM (Z_{DMEM}) and All Trials of Single ESC Suspensions (Z_{EXP}).

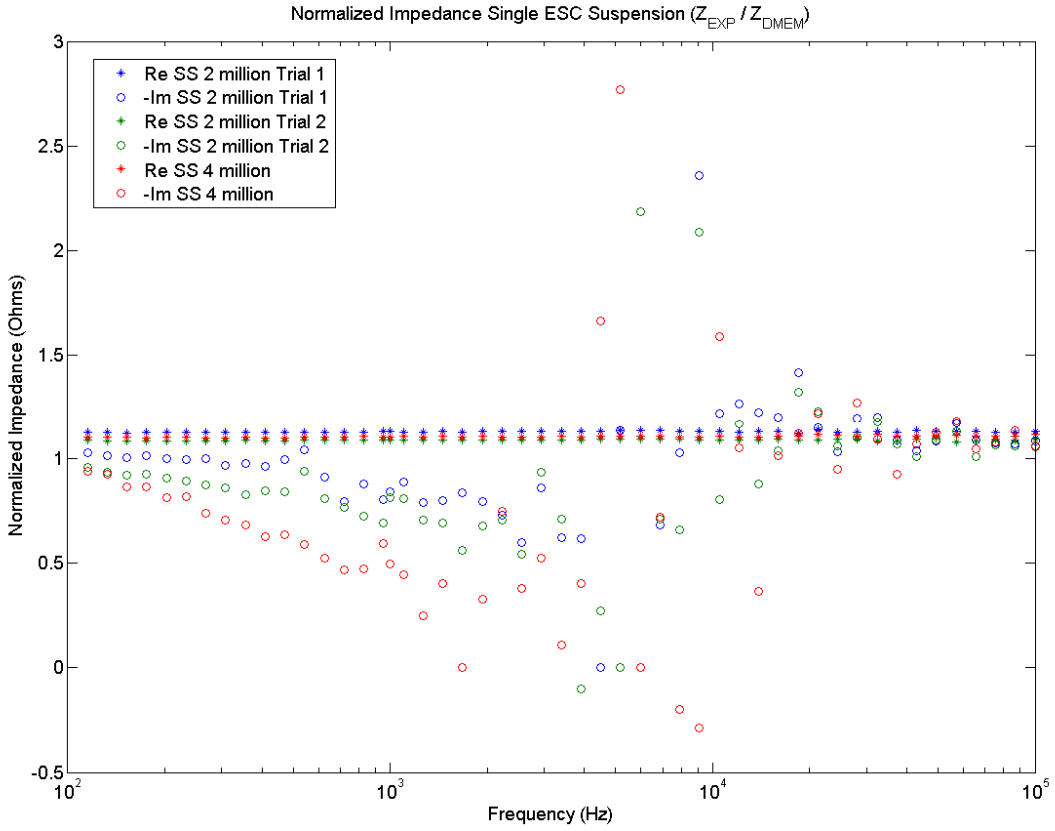

Figure 18 Normalized Impedance Response (Z_{EXP} / Z_{DMEM}) of ESCs in Single Cell Suspension 0.5 mL DMEM.

3.3 Day 4 EBs

After single cell suspensions were analyzed 4 day old (D4) EBs were also investigated. The same experimental protocol was followed. Measurements of different EB densities were made with two trials each of 2,000, 4,000, and 7,000 D4 EBs in DMEM having a total volume of 0.5 mL. Figure 19 shows the complex impedance responses of all the D4 EB trials Z_{EXP} and Z_{DMEM}. The normalized real and imaginary impedance responses, Z_{EXP} / Z_{DMEM}, of each EB density are shown in Figure 20, Figure 21, and Figure 22. Note that in both Figure 21 and Figure 22 for 4,000 and 7,000 EBs each trial differs only by a vertical shift. In Figure 22 however, the real response of trial 1 is larger than trial 2, but the imaginary response is less. This does not

follow the same trend as the other 2 experiments for 4,000 and 2,000 EBs. For the most part, when comparing the different EB concentrations, the real responses are similar in magnitude and shape making it difficult to formulate any obvious conclusions, further data processing is necessary.

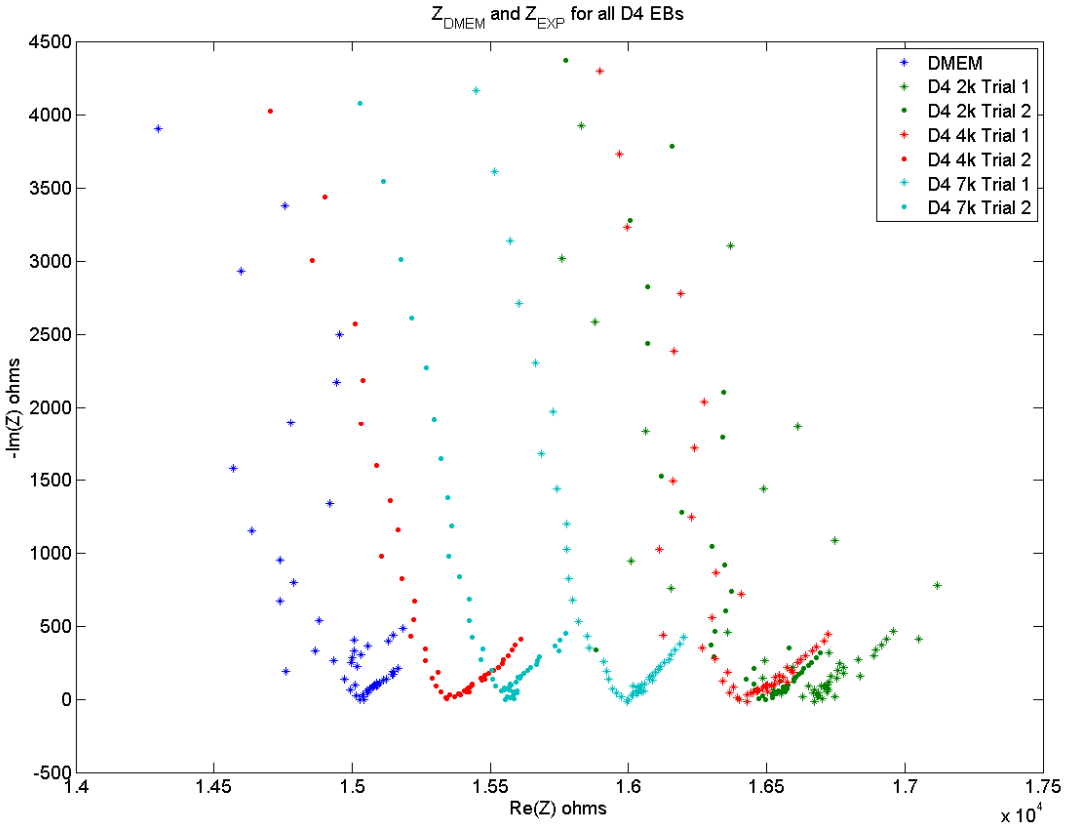

Figure 19 Complex Impedance Response for DMEM (Z_{DMEM}) and all D4 EB Trials (Z_{EXP}).

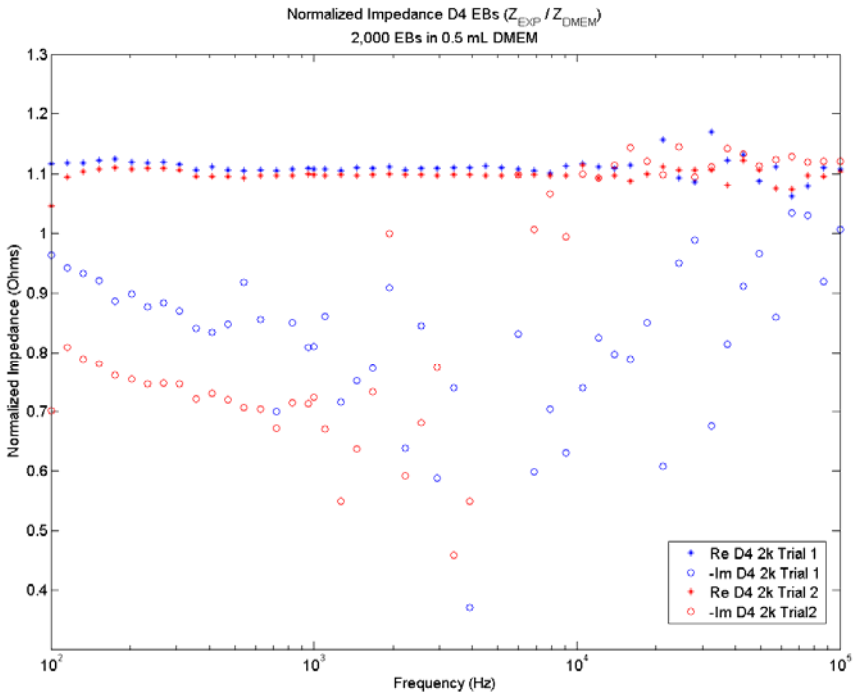

Figure 20 Normalized Impedance Response (Z_{EXP} / Z_{DMEM}) of 2,000 D4 EBs.

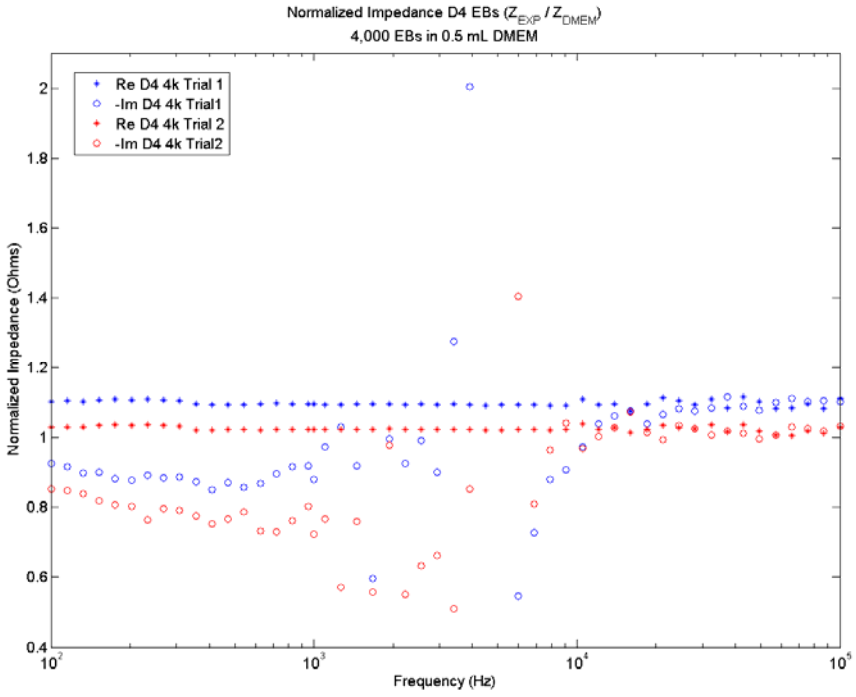

Figure 21 Normalized Impedance Response (Z_{EXP} / Z_{DMEM}) of 4,000 D4 EBs.

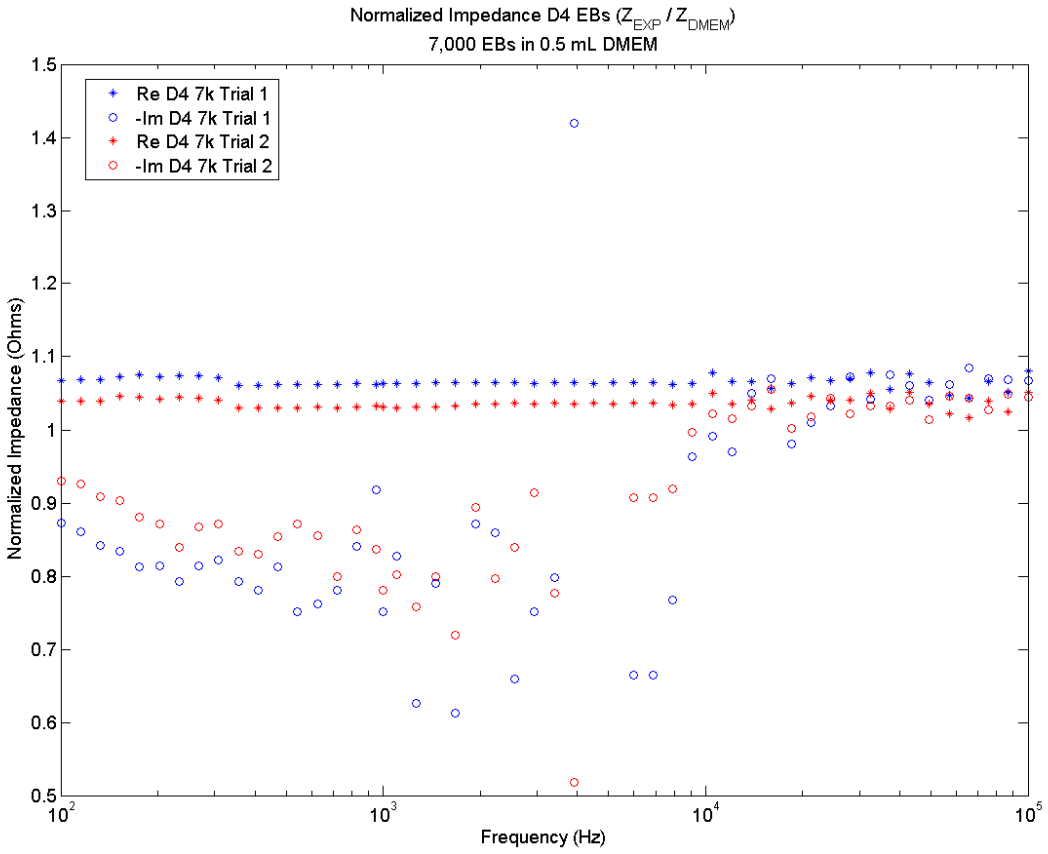

Figure 22 Normalized Impedance Response (Z_{EXP} / Z_{DMEM}) of 7,000 D4 EBs.

CHAPTER 4

DISCUSSION OF RESULTS

Three different types of experiments were conducted that examined the impedance response of undifferentiated media, single cell suspensions of ESCs and D4 EBs from a 1 Vpp signal across a frequency range of 100 Hz to 100 kHz. The conductivity of DMEM media was measured to be 0.5958 S/m. For the single cell suspensions of ESCs it was shown that when 4 million cells are suspended in 0.5 mL of media, the normalized imaginary response decreases with respect to a density of 2 million suspended ESCs which can be a result of the cellular capacitance. Thus cellular presence could be detected and possibly a change in cellular density can be detected. The small change in normalized real response can be attributed to the fact that the media volume dominates the measurement and thus does not change the response.

For D4 EBs, it is hard to discern a specific change in impedance response between the different EB counts of 2,000, 4,000 and 7,000. The normalized real and imaginary impedance responses for the different EB concentrations are similar in magnitude and shape. But, for each trial in the 4,000 and 7,000 experiments, the response shapes are simply vertical shifts of one another, denoting good reproducibility. When comparing the ESC suspension impedance response to D4 EB response, from Figure 17 and Figure 19, the ESC experiments have the same general shape and location as D4 2k EBs. This could be attributed to the volume of 2,000 D4 EBs is similar to the volume of 2 million ESCs.

After examining the experimental results a few things need to be kept in mind. The ESCs are smaller than the EBs and remain suspended in media throughout the experiments. However, the EBs settle quickly to the bottom of the test device. Figure 23 shows what the ESC and EB

suspensions look like during each experiment. The settled EBs could make realizing their impedance response more difficult since the current flows over instead of through the cells. This observation poses the question, what model best represents what is being measured? We propose that the ESC suspension can be considered as a change to the conductive and dielectric properties of the media, as they are well mixed, dense and evenly distributed throughout the media. Figure 24 shows the proposed model representation. It would then be expected that both the real and imaginary impedance responses should change with ESCs when compared to DMEM.

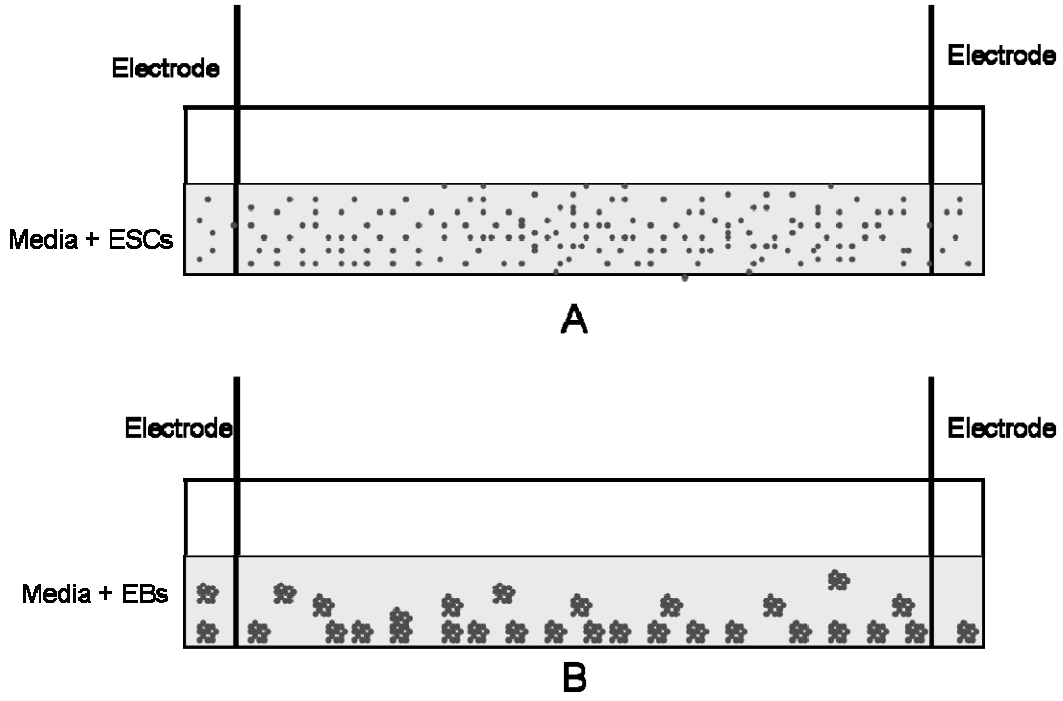

Figure 23 ESCs and EBs During Experimentation. A) ESCs remain suspended B) EBs settle to the bottom.

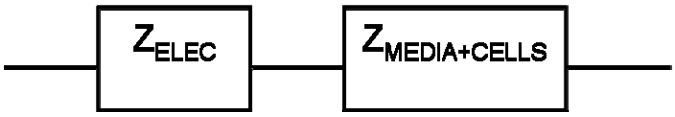

Figure 24 Equivalent Proposed Impedance Model of ESC Suspensions.

The EBs require an alternate test set up, due to their settling, that would make the current flow through the cells, measuring their characteristic instead of the media dominating the measurements. Figure 25 shows an alternative test set up that employs planar electrodes that could be plated in the device. The planar, plated electrodes would allow the current to flow up through the cells and media and make a better measurement. With a new test set up, a new model must be established. Possible models are shown in Figure 26. Figure 24 would not be valid because the EB suspension is not evenly mixed. If there are enough EBs covering the lower electrode, the series model, Figure 26A would be appropriate, however if the electrode is not fully covered, there would be paths between only media or only cells and a parallel model, Figure 26B would be used .

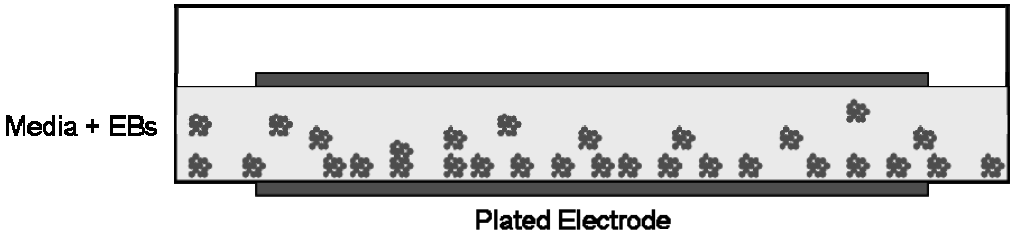

Figure 25 Proposed Re-design of EB Test Set Up with Plated Electrodes.

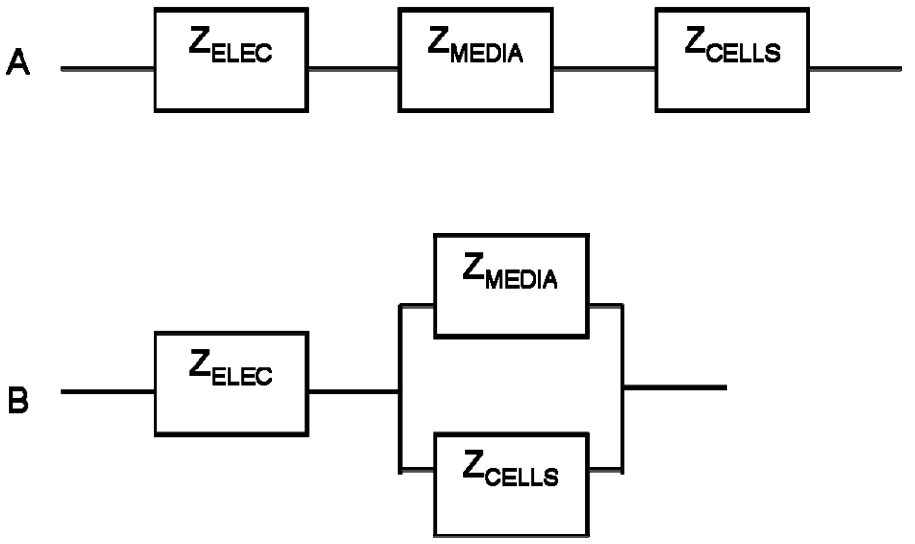

Figure 26 Proposed Equivalent Impedance Model of EB Suspensions A) Series Model B) Media and Cells Parallel Model.

It should be noted that the experiments used cells suspended in 0.5 mL of media, where the 0.5 mL is the total volume of cells and media, and therefore makes it difficult to reliably compare to the measurements taken of 0.5 mL of media alone. For small volumes of ESCs I propose that this should not be a significant effect, but once the volume of ESCs or EBs becomes significant in comparison to the volume of media, there is less media present than the baseline DMEM measurement of 0.5 mL and the two measurements can not be compared. In order to correct this problem, further experiments with the volume of cells added to 0.5 mL of media need to be conducted.

CHAPTER 5

CONCLUSIONS

5.1 Summary of Work

In conclusion, a novel system was designed to examine if ESCs and EBs can be characterized by impedance measurements. The system consists of a polycarbonate channel to hold the cells suspended in media, and a program in Matlab to measure the impedance response due to a 1 Vpp signal across a frequency range of 100 Hz to 100 kHz with the aid of a lock-in amplifier. The system was validated by looking at the impedance response of 1 mL of 0.1 M KCl solution. Then baseline measurements of undifferentiated cell media was looked at followed by experiments with single ESC suspensions of varying cell concentrations in 0.5 mL of media and of D4 EBs at different concentrations in 0.5 mL of media. Initial results from the ESC experiments show promise that cells can be detected when comparing the impedance response of media with cells to that of media alone. A difference in normalized complex impedance response was seen between ESC suspensions containing 2 million cells versus 4 million cells. Little can be said about the impedance response of D4 EBs when comparing varying cell concentrations in 0.5 mL of media, which could be a result of the EBs settling to the bottom of the testing device. Discerning between single ESC suspension impedance responses and D4 EB impedance responses is still inconclusive requiring further experimentation.

5.2 Future Work

This project has many directions it can go for the future. As mentioned in Chapter 4, what was examined used a total cell and media volume of 0.5 mL and compared it to 0.5 mL of media alone. Experiments where the cell volume is added to 0.5 mL of media should be

performed. Higher densities of ESCs and EBs should be looked at, as well as decreasing the amount of media being used because right now, the measurements are dominated by the media and electrode response, instead of the cellular response. EBs at different time periods should be looked at, like D2, D4, and D7 to see if as the EBs increase in size and start to differentiate whether their impedance response changes. EBs subjected to different culture conditions should be tested to see if there are impedance changes that correlate to phenotype changes. The current test set up can be used for the ESC experiments, but a new testing device should be fabricated for the EB experiments, similar to Figure 25. It should hold smaller volumes with electrodes plated on the bottom of the device, to ensure current flowing through EBs instead of over them. With a different testing device design experiments could be done to study the differences between EB populations and individual EBs. If individual EBs can be detected, the system could also be designed to potentially "count" EBs accurately and easily.

Many different aspects of culturing ESCs and EBs have yet to be investigated with this BIM method. The results of this project confirm that the method is feasible. However the testing device and types of experiments need to be altered to have better, useful results.

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